Genome of Methanoregula Boonei 6A8 Reveals Adaptations To Oligotrophic Peatland Environments

By: **Suzanna Brauer**, Hinsby Cadillo-Quiroz, Nikos Kyrpides, Tanja Woyke, Lynne Goodwin, Chris Detter, Sheila Podell, Joseph B. Yavitt, and Stephen H. Zinder

Abstract

Analysis of the genome sequence of Methanoregula boonei strain 6A8, an acidophilic methanogen isolated from an ombrotrophic (rain-fed) peat bog, has revealed unique features that likely allow it to survive in acidic, nutrient-poor conditions. First, M. boonei is predicted to generate ATP using protons that are abundant in peat, rather than sodium ions that are scarce, and the sequence of a membrane-bound methyltransferase, believed to pump Na+ in all methanogens, shows differences in key amino acid residues. Further, perhaps reflecting the hypokalemic status of many peat bogs, M. boonei demonstrates redundancy in the predicted potassium uptake genes trk, kdp and kup, some of which may have been horizontally transferred to methanogens from bacteria, possibly Geobacter spp. Overall, the putative functions of the potassium uptake, ATPase and methyltransferase genes may, at least in part, explain the cosmopolitan success of group E1/E2 and related methanogenic archaea in acidic peat bogs.

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INTRODUCTION

Methanoregula boonei is an acidophilic methanogen isolated from an ombrotrophic peat bog (McLean Bog) in New York State, USA (Bräuer *et al.*, 2006a). A member of the Euryarcheal order *Methanomicrobiales*, this archaeon demonstrates physiological evidence of adaptation to nutrient-poor low ionic strength environments, such as ability to grow at 0.4 mM Na^+ and sensitivity to >50 mM sodium (Bräuer *et al.*, 2011) in contrast to methanogens described elsewhere (Jarrell & Kalmokoff, 1988). As *M. boonei* has been described previously (Bräuer *et al.*, 2011), this paper will focus on a summary of genomic evidence revealing the presence of putative genes specific for proton-rich and sodium- and potassium-poor environments.

M. boonei is the type strain (DSMZ=21154^T, JCM=14090^T) within the type genus of the family *Methanoregulaceae* (Sakai *et al.*, 2012). Cultures are dimorphic, containing thin rods (0.2–0.3 μm in diameter and 0.8–3.0 μm long) and irregular cocci (0.2–0.8 μm in diameter). In PM1 medium, *M. boonei* appears to be an obligate hydrogenotroph and is unable to utilize formate, acetate, methanol, ethanol, 2-propanol, butanol or trimethylamine (Bräuer *et al.*, 2011). Optimal growth conditions are near 35–37 °C and pH 5.1, with growth occurring at pH values as low as 3.8.

METHODS

Preparation of DNA and genome sequencing. *M. boonei* was cultured as described previously (Bräuer *et al.*, 2006b). An exponentially growing culture (1l) was harvested by cold centrifugation and DNA was extracted using a GNOME DNA isolation kit (MP Biomedicals), following the manufacturer's protocols except that a final concentration of 0.1% SDS was added in addition to the cell lysis/denaturing solution to increase cell lysis. Genomic DNA was then evaluated for quality and concentration prior to sequencing.

Table 1. Genome features of *M. boonei* 6A8

Feature	Genome (total)	
	Value	% of total*
Size (bp)	2542943	
No. of G+C bases	1386250	54.5
Coding sequence (bp)	2201702	86.6
Mean ORF length (bp)	893	
5S rRNA	1	
16S rRNA	1	
23S rRNA	1	
tRNA genes	48	
Other RNA genes	3	
Total no. of genes	2518	
Proteins with function prediction	1617	64.2
Proteins without function prediction	847	33.6
GenBank accession no.	NC_009712	

*The total is based on either the size of the genome in bp or the total number of protein encoding genes in the annotated genome.

The genome of *M. boonei* 6A8 was sequenced at the Joint Genome Institute (JGI) using a combination of 3, 8 and 40 kb (fosmid) DNA libraries. All general aspects of library construction and sequencing performed at the JGI can be found at http://jgi.doe.gov/. Draft assemblies were based on 37 430 total reads. All three libraries provided 13 × coverage of the genome. The Phred/Phrap/Consed software package (www.phrap.com) was used for sequence assembly and quality assessment (Ewing & Green, 1998; Ewing *et al.*, 1998; Gordon *et al.*, 1998). After the shotgun stage, reads were assembled with parallel Phrap (High Performance Software). Possible misassemblies were corrected with Dupfinisher (Han & Chain, 2006). Gaps between contigs were closed by editing in Consed or custom primer walk. A total of 921 additional reactions were necessary to close gaps and to raise the quality of the finished sequence. The completed genome sequences of *M. boonei* 6A8 contains 37 526 reads, achieving a mean of 13-fold sequence coverage per base with an error rate of less than 1 in 100 000.

Additional gene functional annotation and comparative analyses were performed within the Integrated Microbial Genomes (IMG/ER) platform (Markowitz *et al.*, 2006). Alignments of functional genes were conducted in BioEdit using CLUSTAL W (Larkin *et al.*, 2007). Phylogenetic trees were reconstructed using the PHYLIP software

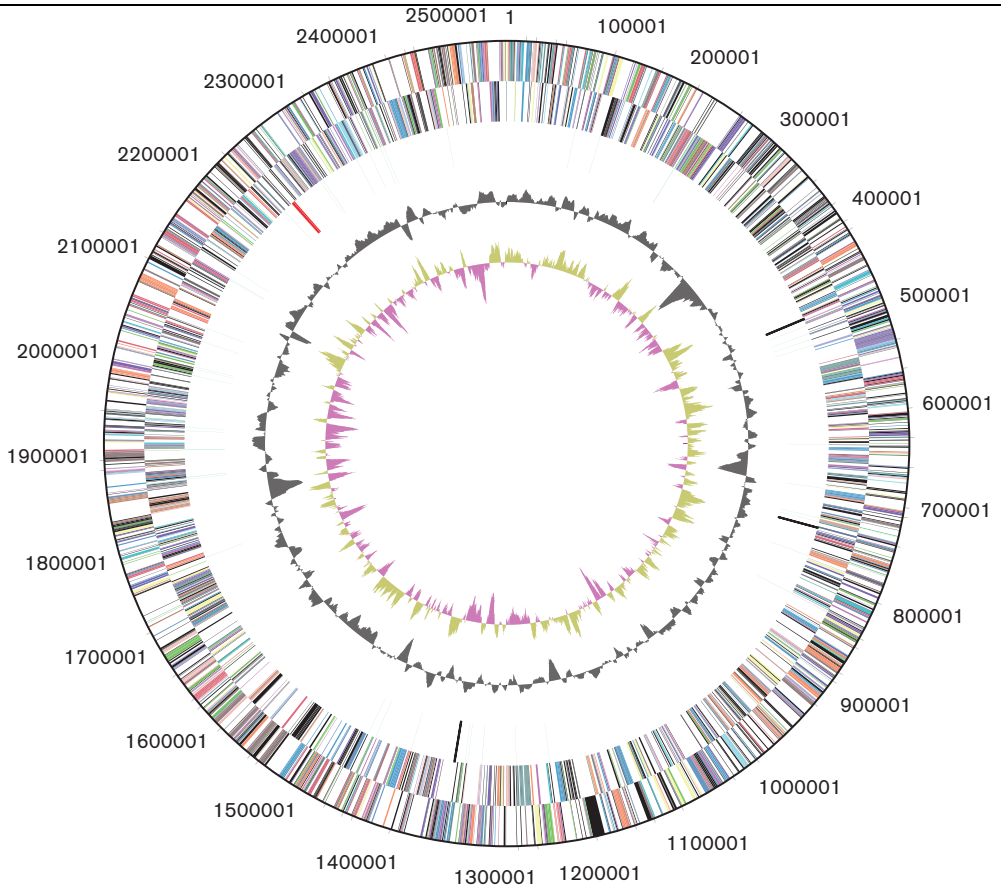

Fig. 1. Circular map of the *M. boonei* 6A8 genome. From the outside ring to the centre: (i) genes on the forward strand, coloured by COG category (Tatusov *et al.*, 2000); (ii) genes on the reverse strand, coloured by COG category; (iii) RNA genes with tRNA genes coloured green, rRNA genes red and other RNAs black; (iv) GC content; (v) GC skew. This figure is available on the IMG website (https://img.jgi.doe.gov/) (Markowitz *et al.*, 2006).

package (Felsenstein, 2004) by conducting both neighbour-joining and maximum-likelihood analysis.

Nucleotide accession number. The complete genome sequence of *M. boonei* strain 6A8 is available in the National Center for Biotechnology Information database (Wheeler *et al.*, 2007) under GenBank/EMBL/DDBJ accession number NC_009712. Additionally, the genome is available in the IMG system (Markowitz *et al.*, 2006) and the JGI genome portal (Grigoriev *et al.*, 2012).

RESULTS AND DISCUSSION

Genome sequencing and annotation information

M. boonei was selected for sequencing due to its potential energy production (methane), biogeochemical importance in global carbon cycling and occurrence in habitats that are unique for cultured methanogens, i.e. proton rich and nutrient element poor. Sequencing, assembly and annotation were conducted by the Department of Energy JGI. A summary of the genome sequencing information can be found in Table 1.

Genome properties

The genome consists of one single circular chromosome of approximately 2.5 million bp with 2518 genes identified, including one rRNA gene operon (Table 1, Fig. 1). Highlighting our dearth of knowledge of methanogenic archaea, only 36% of the genes were associated with one of the well-defined cluster of orthologous groups (COG) categories (Tatusov *et al.*, 2000), with the remaining 64% either not associated with a COG (39%), or associated only by general (14%) or unknown (12%) function (Table 2). The majority of the COG genes in *M. boonei* were predicted to be involved in energy production and conversion (9%), translation (8%), and transport and metabolism of amino-acids (8%), coenzymes (6%) and ions (6%).

Adaptation to high proton concentrations

In McLean Bog, the pH is near 4 and the H^+ concentration is approximately 10^{-4} M (100 μM), three orders of magnitude greater than at pH 7. Moreover, sphagnum moss impedes the flow of mineral-rich groundwater into the bog so that the only water source is rain, essentially distilled water. Consequently, Na^+ concentrations are typically low. For example, Na^+ concentrations were measured as only 2 μM in the McLean Bog porewater (Bräuer *et al.*, 2004). These exceedingly low external Na^+ concentrations make developing and conserving a sodium motive force challenging; however, sodium motive force is considered essential to energy conservation by methanogens (Schlegel & Müller, 2013).

All cultured members of the groups *Methanobacteriales*/*Methanococcales* lack cytochromes, and the only known energy-conserving step is Na^+ pumping coupled to methyl group transfer by the membrane-bound enzyme complex methyltetrahydromethanopterin : coenzyme M methyltransferase (Mtr) (Schlegel & Müller, 2013; Thauer *et al.*, 2008). Further, the A_1A_0 ATPase/synthases studied among the members of the groups *Methanobacteriales*/*Methanococcales* have clearly been shown to pump Na^+ (McMillan *et al.*, 2011; Mulkidjanian *et al.*, 2008). In contrast, the cytochrome-containing methanogens in the *Methanosarcinales* have Mtr complexes, but also have steps that pump protons (Schlegel & Müller, 2013; Thauer *et al.*, 2008), and evidence has favoured H^+ pumping by the ATPases in these organisms (Müller *et al.*, 1999; Pisa *et al.*, 2007). More recently, it was demonstrated that the A_1A_0 archaeal ATPase/synthase in *Methanosarcina acetivorans* is 'promiscuous', pumping either Na^+ or H^+ (Schlegel *et al.*, 2012) with both ions possible at neutral pH, especially at seawater salinity of 0.4 M Na^+, whereas only protons were pumped at pH 5. *M. boonei* belongs to the *Methanomicrobiales*, which lack cytochromes like the *Methanobacteriales*/*Methanococcales* cluster, but are more closely related to the *Methanosarcinales*, and it is not clear which patterns of bioenergetics this group follows.

It is the AtpC/K subunit of the membrane bound A_0 subunit of the A_1A_0 ATPase/synthase complex that is responsible for

Table 2. Genes associated with COG functional categories

COG category	No. of genes	% of total*
Energy production and conversion	157	9.35
Translation, ribosomal structure and biogenesis	134	7.98
Amino acid transport and metabolism	133	7.92
Coenzyme transport and metabolism	108	6.43
Inorganic ion transport and metabolism	96	5.71
Signal transduction mechanisms	95	5.65
Transcription	83	4.94
Post-translational modification, protein turnover, chaperones	69	4.11
Replication, recombination and repair	69	4.11
Carbohydrate transport and metabolism	61	3.63
Cell wall/membrane/envelope biogenesis	55	3.27
Nucleotide transport and metabolism	53	3.15
Cell motility	28	1.67
Defence mechanisms	27	1.61
Lipid transport and metabolism	24	1.43
Intracellular trafficking, secretion and vesicular transport	22	1.31
Cell cycle control, cell division, chromosome partitioning	18	1.07
Secondary metabolites biosynthesis, transport and catabolism	15	0.89
Chromatin structure and dynamics	2	0.12
General function prediction only	227	13.51
Function unknown	204	12.14
Not in a COG category	971	38.56

*The total is based on the total number of protein encoding genes in the annotated genome.

pumping either H^+ or Na^+, with the ion typically binding to a conserved aspartate or a glutamate located within a transmembrane helix. In Table 3, partial sequences of the AtpC/K are aligned, and residues critical to Na^+ binding in the *Methanobacteriales/Methanococcales* (Grüber *et al.*, 2014; McMillan *et al.*, 2011) are indicated in bold. Also shown in bold are residues in the *M. acetivorans* sequence that are considered crucial to being able to bind either Na^+ or H^+. The *M. boonei* sequence shares these residues as well as many others with *M. acetivorans*, and it is likely that its ATPase pumps protons near pH 4.

Another question of interest is whether the Mtr complex in *M. boonei* pumps Na^+. In *Methanosarcina mazei* and *Methanothermobacter marburgensis*, Na^+ pumping was demonstrated for the Mtr complex, thereby leading to a sodium motive force. From these results, it was extrapolated that all Mtr complexes pump Na^+ (Schlegel & Müller, 2013). The pumping is attributed to the membrane-bound MtrE subunit, and a specific aspartate (indicated by the bold D) predicted to be within a transmembrane helix, part of the motif 168-IWGITIGAIGSSTGDVHYGAER-191 that is conserved between the two organisms (Gottschalk & Thauer, 2001). *M. boonei* and other members of the *Methanomicrobiales* have an asparagine instead of aspartate at that position (position 190 in Fig. S1, available in the online Supplementary Material) in their MtrE sequences, which renders that residue unable to pump cations. There is a glutamate at position 253 in Fig. S1 within a region (predicted to be a transmembrane alpha helix by the IMG website) that is conserved amongst *Methanomicrobiales* as well as some other methanogens, but is not present in the *M. marburgensis* or *Methanosarcina barkeri* sequences. This residue may play a role in pumping but, as of now, it is

Table 3. Amino acid alignment of AtpCK demonstrating the two conserved glutamine (Q) and tyrosine (Y) residues (shown in bold) (identified by: McMillan *et al.*, 2011; Mulkidjanian *et al.*, 2008; Sakai *et al.*, 2011) that appear to be unique for methanogens predicted to have sodium-driven ATPases versus those predicted to have proton-driven or sodium-proton driven ATPases

M. boonei is shaded in grey. Organisms predicted to have proton-driven ATPases are shown in red font and those predicted to have sodium-driven ATPases are shown in blue font. Organisms with experimental evidence supporting sodium-driven ATPases are shown in bold blue and include: *Methanococcus jannaschii* (Morsomme *et al.*, 2002), *Methanococcus voltae* (Dybas & Konisky, 1992), *Methanobacterium thermoautotrophicum* (Schönheit & Perski, 1983) and *Methanobrevibacter ruminatum* (McMillan *et al.*, 2011). Organisms with experimental evidence supporting proton-driven (or sodium/proton-driven) ATPases are shown in bold red and include: *M. mazei* Gö1 (Becher & Müller, 1994; Pisa *et al.*, 2007), *Methanosaeta thermophila* (Inatomi *et al.*, 1993) and *M. barkeri* (Blaut & Gottschalk, 1984; Müller *et al.*, 1999). *M. acetivorans* C2A was recently shown to have a sodium-proton driven ATPase (Grüber *et al.*, 2014; Schlegel & Müller, 2013) and the residues considered crucial to being allowing binding of both ions are indicated in bold, as are the corresponding amino acids in the *M. boonei* sequence directly above. IMG gene numbers follow the colon. Blue shading has been added to indicate sequences that align with those of organisms predicted to have sodium-driven ATPases and red shading indicates sequences that align with those of predicted proton-driven ATPases.

Organism	Partial amino acid alignment						
Methanospirillum hungatei JF-1:637896821	KAVGAGLAVG	LAGVGSGLGE	MGIGAAAMGA	VAENKDMFGL	ALLFTVLPET	IVIFGLVVAL	LL
Methanospirillum hungatei JF-1:637897383	VPIGAAIAFA	GGAIATGIAQ	SKIGAAGAGT	VAERPESAGT	VIVLEAIPET L	VILGFVVAA	MI
***Methanosarcina mazei* strain Gö1:638165281**	KALGAAIAIA	VTGLASAIAE	KDIGTAAIGA	MAENEGLFGK	GLILTVIPET	IVIFGLVVAL	LI
Methanoregula boonei 6A8:640869605	KAIGAGLAVG	LTGVGTGVA**E**	MGIGAAAVGA	IAENKDFFGL	GLLFTV**IPET**	**IVIF**GLVIAL	LL
***Methanosarcina acetivorans* C2A: 638179041**	KALGAALAIT	VTGLASAWA**E**	KEIGTAAIGA	MAENEGLFGK	GLILTV**IPET**	**IVIF**GLVVAL	LI
***Methanosarcina barkeri* str. fusaro: 637699281**	KAIGASIAIA	LTGIASAIAE	KDIGTAAIGA	MAENEGLFGK	GLILTVIPET	IVIFGLVVAL	LI
Methanoculleus marisnigri JR1:640114955	SAVGAGLAVG	LTGVGTGLAE	MGIGAAAVGA	TAENRDMFGL	ALLFTVIPET	IVIFGLVVAL	LL
Methanosphaerula palustris E1-9c: 643571272	KAVGAGLAVG	LAGIGTGLGE	MGIGAAAMGA	TAENKDMFGL	ALLFTVIPET	IVIFGLVVSL	LL
Methanocella paludicola SANAE: 646465407	VAIGAGLAVG	LAGIGSGIAE	KDIGAAAVGA	IAEDRSFFGQ	GLIFTVIPET	IVIFGLVIAI	LL
Methanosaeta concilii GP6:650798510	IAVGAGLATG	LAGIGAGVGE	QGIGAAVVGV	VAEEPGFLGK	GLFLMLLPET	LIIFGLAVSL	IL
Methanocella paludicola SANAE: 646467386	IPLGAAIAFG	AGAISTGFAQ	ARIGSAGAGA	LSERPELSGL	IIILEAIPET	LAILGFVVAA	MI
Methanohalophilus mahii DSM 5219:646707499	KAIGAGLAVG	LTGLASGIAE	KDIGAAAIGA	MAENEGLFGK	GLIMTVIPET	IVIFGLVVAL	LI
Methanobacterium sp. AL-21:650750551	AAIGAGLAVG	LAGLGSGIGQ	GIAAAGSVGA	VAEDPDMFAR	GIIFTALPET	**QAIY**GFLIAI	LL
Methanobacterium sp. SWAN-1:650872332	AAIGAGVAVG	FAALGSGIGQ	GIASAGAVGA	VAEDKSMFAQ	GMVFTAIPET	**QAIY**GFLISI	LL
Methanopyrus kandleri AV19:638169043	AAIGAGLAAG	VAGVGSGIGQ	GIAAAAGAGA	VAEDEATFGK	AIVFSVLPET	**QAIY**GLLTAI	LI
***Methanocaldococcus jannaschii* DSM 2661:638201515**	GAVGAGLAVG	IAGLGSGIGA	GITGASGAGV	VAEDPNKFGT	AIVFQALPQT	**QGLY**GFLVAI	LI
Methanospirillum hungatei JF-1:637897394	MAIGAGIAVG	CSAIGSGIGV	GIVGSAASGV	ISERSEKFGM	ALVFTAIPQT	**QAIY**GLLIAI	LI
***Methanothermobacter thermoautotrophicus*: 638155490**	AAIGAGVAVG	FAGLGSGLGQ	GIAAAESVGA	VAENSDMFAR	GIIFSTLPET	**QAIY**GFLIAI	LL
Methanococcus maripaludis C5:640166057	GAIGAGLAVG	IAGLGSGIGA	GITGASGAGV	VAEDPNKFGT	AIVFQALPQT	**QGLY**GFLVAI	LI
Methanobrevibacter smithii DSM 2375:644143574	AAIGAGVAIG	FAGLGSGLGQ	GMAAAGSVGA	VAEDNDMFAR	GIIFSALPET	**QAIY**GFLIAI	LL
Methanosphaera stadtmanae DSM 3091:637847029	AAIGAGVAVG	FAALGSGIGQ	GIASSASVGA	VAEDSSMFAQ	GLVFTAIPET	**QAIY**GFLIAI	LL
Methanobrevibacter smithii ATCC 35061:640592228	AAIGAGVAIG	FAGLGSGLGQ	GMAAAGSVGA	VAEDNDMFAR	GIIFSALPET	**QAIY**GFLIAI	LL
***Methanobrevibacter ruminantium* M1:646531773**	AAIGAGVAIG	FAGLGSGLGQ	GMAAAGSVGA	VAEDNDMFAR	GIIFSALPET	**QAIY**GFLIAI	LL
***Methanococcus voltae* A3:646858602**	GAIGAGLAVG	IAGLGSGIGA	GITGASGAGV	LAEDPKQFSK	VIVFQALPQT	**QGLY**GFLVAI	LI
Methanotorris igneus Kol 5:650856311	GAIGAGLAVG	IAGLGSGIGA	GITGASGAGV	VAEDPNKFGT	AIVFQALPQT	**QGLY**GFLVAI	LI
Methanothermococcus okinawensis IH1:650918276	GAVGAGLAVG	IAGLGSGIGA	GITGASGAGV	VAEDPNKFGT	AIVFQALPQT	**QGLY**GFLVAI	LI

unclear whether the Mtr complex in *M. boonei* or other *Methanomicrobiales* pumps Na^+ or H^+, or perhaps is not a pump at all. Because of these fundamental differences in the MtrE sequences between *Methanomicrobiales* and other methanogens, the role of Mtr in their bioenergetics warrants examination, especially since it is considered the only site for energy conservation in these organisms.

Adaptation to low potassium concentrations

Similar to the case for Na^+, the K^+ concentrations in McLean Bog porewater are extremely low, less than 25 μM (Bräuer *et al.*, 2004), and cells typically accumulate K^+ (Epstein, 2003) as well as expel Na^+. *M. boonei* is predicted to carry genes for three different K^+ uptake mechanisms including the low-affinity *trk* genes that many methanogens carry, in addition to the medium-affinity *kup* genes and the ATP-driven high-affinity *kdp* genes, both of which are more rarely found among methanogenic archaea (Table S1). Only one other methanogen (sequenced to date) carries all three predicted K^+ uptake systems, *Methanosphaerula palustris* E1-9c, and it was also isolated from a peatland ecosystem (Cadillo-Quiroz *et al.*, 2009; Cadillo-Quiroz *et al.*, 2008), a fen in which the pH was neutral but the K^+ porewater concentrations were only 3–8 μM (Dettling *et al.*, 2007).

In *Escherichia coli*, the *kdp* uptake system shows both high specificity and high affinity for potassium, and is required for growth during extreme potassium limitation (Altendorf & Epstein, 1996; Epstein, 2003; Epstein *et al.*, 1990). *E. coli* cultures with a mutation in the *kdp* genes have shown growth deficiencies at K^+ concentrations below 300 μM (Rhoads *et al.*, 1976). Highlighting its importance in *M. boonei*, the *kdpCAB* operon has been duplicated and can be identified in two locations in the genome (Fig. 2). Compared to *E. coli*, both predicted KdpA proteins in *M. boonei* (Mboo 0443 and 0894) have all four regions (I, 112-NTNWQ-116; II, 230-TNGGG-234; III, 343-SCGAV-347; IV, 468-NNGSA-472; *E. coli* numbering) demonstrated experimentally (Bertrand *et al.*, 2004; Buurman *et al.*, 1995; Dorus *et al.*, 2001; Schrader *et al.*, 2000; van der Laan *et al.*, 2002) and in 3D structural models (Greie, 2011; Hu *et al.*, 2008) to be responsible for K^+ binding. Originally identified by the HGT-detection program, DarkHorse (Podell & Gaasterland, 2007), the predicted KdpA proteins in *M. boonei* and in other methanogens cluster phylogenetically within the *Proteobacteria*, perhaps most closely resembling those of *Geobacter* spp. (Fig. 3). Since there are apparently three closely related clades of methanogen KdpA protein sequences, it is unclear how many transfer events have occurred. Moreover, the KdpC protein is predicted to be fused to the N-terminal of KdpA (Fig. 2) in both sets of genes, an arrangement shared with *Methanomassiliicoccus luminyensis*, a methanogen isolated from human faeces (Dridi *et al.*, 2012) belonging to a new phylum related to *Thermoplasma* and only able to use H_2 and methanol for methanogenesis. All other methanoarchaea, including the closely related *Methanosphaerula*, have the canonical

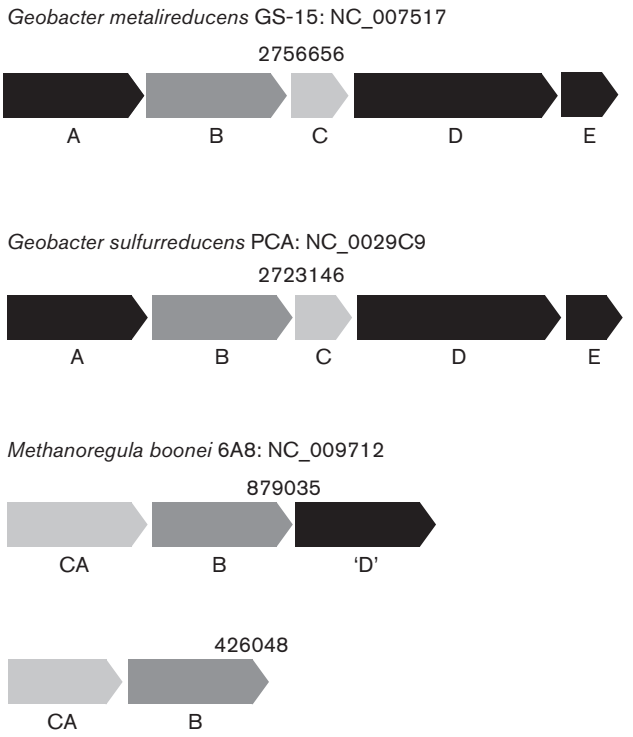

Fig. 2. Diagram of high affinity, ATP-driven potassium uptake (*kdp*) gene arrangement in *Geobacter* spp., which shows the canonical arrangement, compared to the two *kdp* operons [Mboo 0894-6 (top) and Mboo 0443-4 (bottom)] in *M. boonei* 6A8. This figure was modified from an image on the IMG website (https://img.jgi.doe.gov/) (Markowitz *et al.*, 2006). Genes encoding for KdpA, KdpB, KdpC, KdpD or KdpE subunits are indicated by an A, B, C, D or E, respectively. The fused *kdpC/A* gene is indicated by a CA and the predicted pseudogene *kpdD* is indicated by a 'D'.

kdpABC gene order. Thus, the arrangement and close phylogenetic relationship of their *kdpCA* genes relative to that of other organisms suggests that a gene transfer event occurred between ancestors of *M. boonei* and *M. luminyensis*.

In *E. coli* and many other bacteria, KdpD is a membrane-bound osmosensitive K^+-sensing histidine kinase component, and KdpE is the response regulator of a two-component transcriptional regulatory system that induces *kdp* genes when K^+ is low (Nakashima *et al.*, 1992; Poolman & Glaasker, 1998) (Fig. 2). A number of methanoarchaea with *kdp* genes possess a *kdpD* gene (Table S1). In *M. boonei*, the *kdpD* gene is predicted to encode a truncated protein lacking the histidine kinase domain (Table S2) and to also lack *kdpE* compared to bacteria. Thus, it is unlikely that KdpD is a transcriptional regulator and it may play some other role in regulating activity of the Kdp or other proteins, since it still maintains membrane-bound sensing domains. Some *Bacteria*, including *Cyanobacteria* and *Deinococcus radiodurans*,

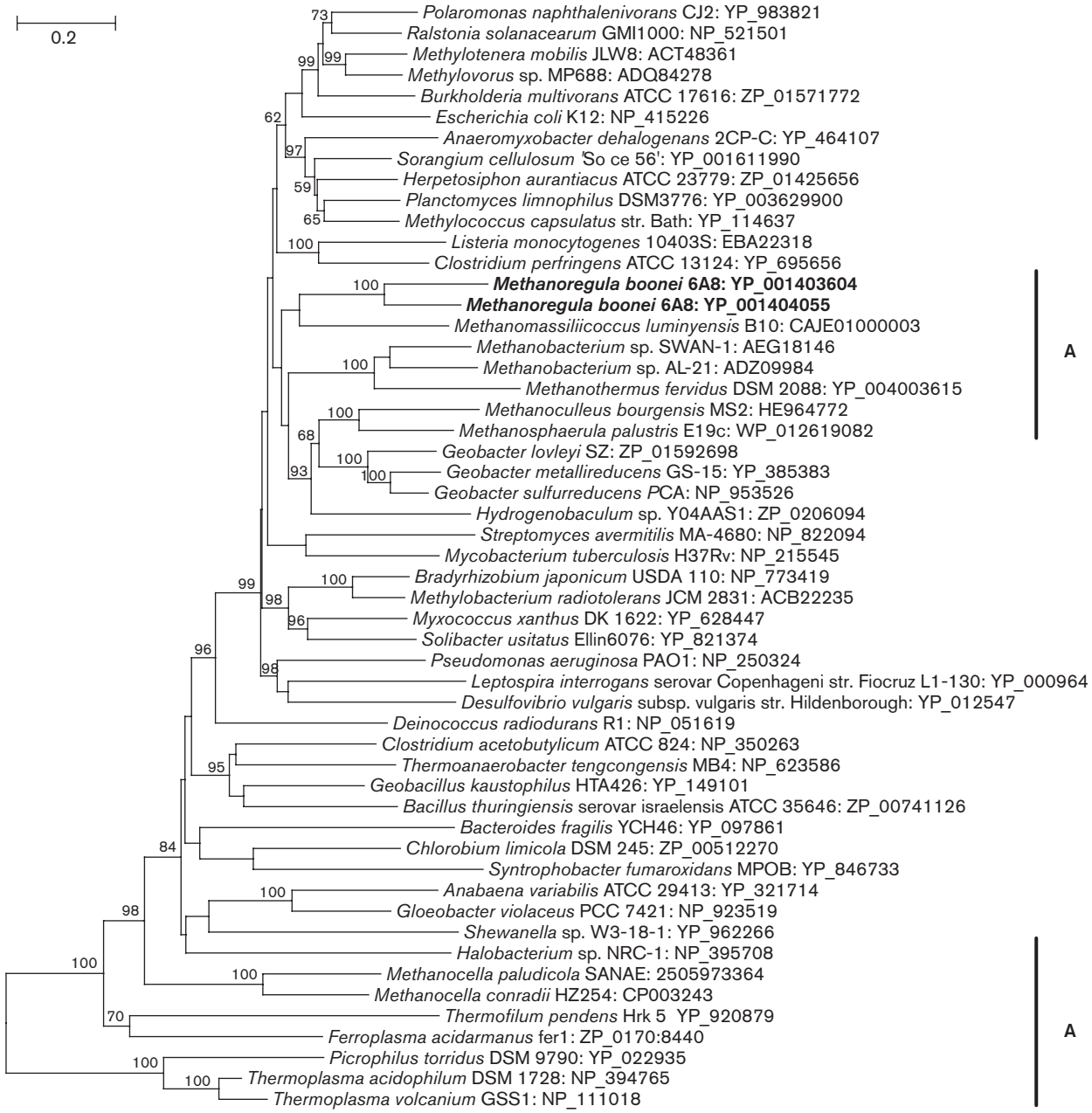

Fig. 3. Neighbour-joining dendrogram of KdpA. The tree was rooted using deep-branching members of the *Thermoplasmatales*. Archaeal sequences are indicated with an A. Accession numbers or IMG gene numbers follow the colon. Bootstrap values greater than 60 are shown for nodes that were supported by maximum-likelihood analysis. *M. boonei* is shown in bold. The scale bar indicates the number of protein changes per site.

also contain a truncated *kdpD* and lack *kdpE* (Ballal *et al.*, 2007). Although *kdpF* is also absent, this gene is non-essential for potassium transport *in vivo*, according to studies in *E. coli* (Gaßel *et al.*, 1999).

Similarly, the *kup* genes may have also been horizontally transferred between *Geobacteraceae* and *Methanosarcina* spp. and several members of the *Methanomicrobiales* (Fig. 4). However, the data are less robust since the *Geobacter* spp. KupA protein sequences do not cluster with other *Proteobacteria*, so the direction of transfer is unclear. Still, it's most likely that the methanoarchaeal KupA sequences were derived from *Bacteria*, according to the phylogenetic clustering (Fig. 4). Essentially all of the methanoarchaea possess low-affinity potassium uptake genes (*trk*; Table S1); thus, these genes will not be discussed.

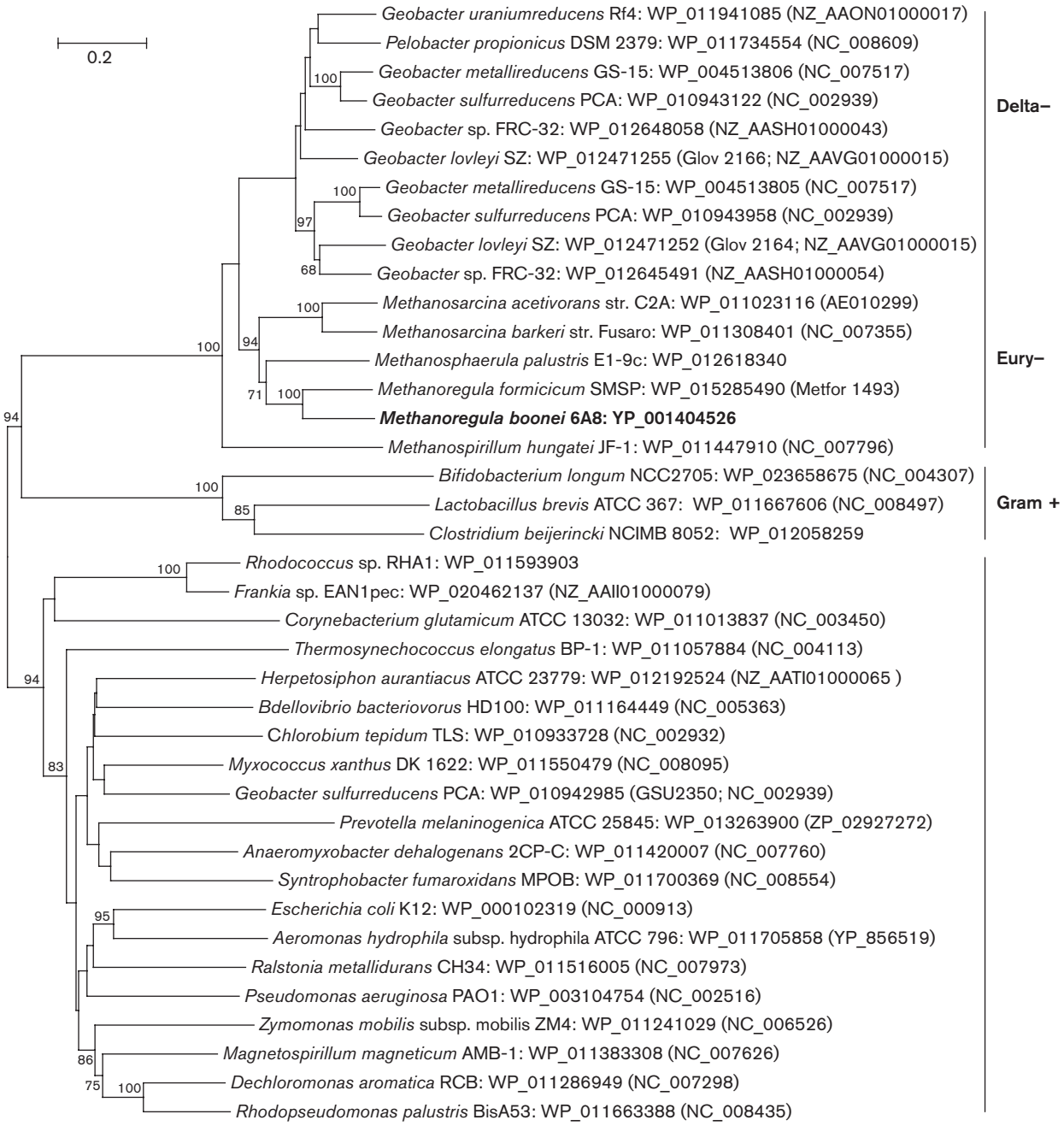

Fig. 4. Unrooted neighbour-joining dendrogram of the predicted KupA protein in *M. boonei* compared to that of other organisms. Several distinct clusters are shown including: one that contains members of the phyla *Cyanobacteria*, *Choroflexi*, *Chlorobi*, *Proteobacteria* and *Actinobacteria* (unlabelled); one that includes Gram-positive *Actionobacteria* and *Firmicutes* (Gram+); and one that includes *Proteobacteria* of the genus *Geobacter* (Delta−), as well as members of the *Methanomicorbia* class of the *Euryarchaeota* (Eury−). Bootstrap values greater than 60 are shown for nodes that were supported by maximum-likelihood analysis. *M. boonei* is shown in bold. The scale bar indicates the number of protein changes per site.

CONCLUSION

Organisms related *to M. boonei* in the E1/E2 cluster, R10, or fen cluster are widespread throughout acidic to moderately acidic peatlands in Germany (Hamberger *et al.*, 2008; Wüst *et al.*, 2009), England (Edwards *et al.*, 1998; Hales *et al.*, 1996), Russia (Kotsyurbenko *et al.*, 2007), Scandinavia (Galand *et al.*, 2005; Høj *et al.*, 2005), the United States (Basiliko *et al.*, 2003; Cadillo-Quiroz *et al.*, 2006; Hawkins *et al.*, 2014) and Canada (Godin *et al.*, 2012; Yavitt *et al.*,

2006). Further, this group tends to dominate in ombrotrophic bogs and is often outcompeted in minerotrophic fens, where the methanogenic community becomes more diverse (Galand *et al.*, 2005; Kotsyurbenko, 2010; Kotsyurbenko *et al.*, 2007). For example, microbial diversity was shown to increase along a gradient from pH 4.2 in an ombrotrophic bog to 5.1 in a mesotrophic fen in Finland (Juottonen *et al.*, 2005). Similarly, a fen in Minnesota was found to have higher diversity than that of a nearby bog (Lin *et al.*, 2012). The genome of *M. boonei* harbours evidence of adaptation to a proton-rich, sodium-poor and potassium-poor environment, which may, in part, explain the cosmopolitan success of this and related organisms in acidic peat bogs.

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